

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
22 March 2001 (22.03.2001)

PCT

(10) International Publication Number
WO 01/20044 A3

(51) International Patent Classification?: C12Q 1/68,
G01N 15/14

(21) International Application Number: PCT/US00/25465

(22) International Filing Date:
15 September 2000 (15.09.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/154,601 17 September 1999 (17.09.1999) US

(71) Applicant (for all designated States except US): THE
GOVERNMENT OF THE UNITED STATES OF
AMERICA, as represented by THE SECRETARY,
DEPARTMENT OF HEALTH & HUMAN SER-
VICES, THE NATIONAL INSTITUTES OF HEALTH
[US/US]; Office of Technology Transfer, Suite #325, 6011
Executive Boulevard, Rockville, MD 20852 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): KALLIONIEMI,

Olli, P. [FI/US]; 1083 Grand Oak Way, Rockville, MD
20852 (US). KONONEN, Juha [FI/US]; 5801 Nicholson
Lane, Apartment #1021, Bethesda, MD 20852 (US).
BUDENDORF, Lukas [CH/US]; dROSSELSTRASSE
31, CH-4106 Therwil (US). DOUGHERTY, Edward,
R. [US/US]; 3101 Westchester Avenue, College Station,
TX 77845 (US). GRIGORYAN, Artyom, M. [AM/US];
11246 Cedar Park, San Antonio, TX 78249-4263 (US).

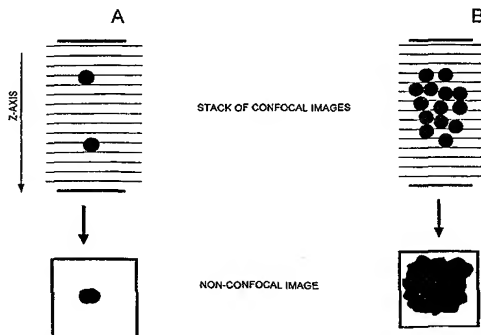
(74) Agent: NOONAN, William, D.; Klarquist, Sparkman,
Campbell, Leigh & Winston, LLP, One World Trade Cen-
ter, Suite 1600, 121 SW Salmon Street, Portland, OR 97204
(US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ,
DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR,
HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,
LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,
NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM,
TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European

[Continued on next page]

(54) Title: SIGNAL COUNTING FOR IN SITU HYBRIDIZATION



(57) Abstract: Fluorescently tagged nucleic acid probe signals are counted in biological specimens by determining a ratio of signals from a test probe to signals of a reference probe. Probe signals need not be counted with reference to cells, nuclei, or nuclear contours. Gene amplification or deletion can thus be detected by analyzing the ratio. Successive image slices are obtained by confocal microscopy, and the images are digitized. The digital images are transformed and analyzed to combine contiguous fluorescent signal segments in successive optical sections to identify discrete probe signals, or spots. Spots overlapping in the axial and transverse dimensions of a three-dimensional representation of the biological specimens can be distinguished. A graphical user interface presents various features for consideration by a user, who can provide guidance to a computer system counting the spots. Various features directed to identifying spot clusters and autofluorescent material can increase accuracy of spot counting.

RECEIVED
FEB 13 2003
TECH CENTER 1600/2900

WO 01/20044 A3



patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(88) Date of publication of the international search report:
10 May 2002

Published:

— with international search report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/US 00/25465

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 C12Q1/68 G01N15/14		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 7 C12Q		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RODENACKER ET AL.: "Groping for quantitative digital 3-D image analysis: an approach to quantitative fluorescence in situ hybridization in thick tissue sections of prostate carcinoma" ANALYTICAL CELLULAR PATHOLOGY, vol. 15, no. 1, 1997, pages 19-29, XP001024113	1,6-23, 27-40, 48-63
Y	the whole document	1-26, 41-47
X	WO 99 44063 A (THE UNITED STATES OF AMERICA) 2 September 1999 (1999-09-02)	1-5
Y	page 13, line 16 -page 24, line 20; examples 1,3	1-14, 24-26, 41-47
--- -/-		
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. "Z" document member of the same patent family		
Date of the actual completion of the international search 17 September 2001		Date of mailing of the international search report 21/09/2001
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040. Tx. 31 651 epo nl. Fax: (+31-70) 340-3016		Authorized officer Botz, J

IN. NATIONAL SEARCH REPORT

Inter. onal Application No
PCT/US 00/25465

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AUBELE ET AL.: "Comparative FISH analysis of numerical chromosome 7 abnormalities in 5-um and 15-um paraffin-embedded tissue sections from prostatic carcinoma" HISTOCHEMISTRY AND CELL BIOLOGY, vol. 107, no. 2, 1997, pages 121-126, XP001024090	1,6-23, 27-40, 54-63
Y	the whole document	1-14, 24-26, 41-47
Y	US 5 817 462 A (GARINI ET AL.) 6 October 1998 (1998-10-06) column 26, line 45 - line 58	1-23

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/25465

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9944063	A	02-09-1999	AU 2875799 A	15-09-1999
			AU 2973599 A	15-09-1999
			EP 1068528 A2	17-01-2001
			EP 1066517 A1	10-01-2001
			WO 9944062 A1	02-09-1999
			WO 9944063 A2	02-09-1999
US 5817462	A	06-10-1998	US 5936731 A	10-08-1999
			US 5784162 A	21-07-1998
			US 5539517 A	23-07-1996
			AU 2730597 A	12-11-1997
			DE 29724412 U1	22-03-2001
			EP 0896631 A1	17-02-1999
			JP 2000509977 T	08-08-2000
			WO 9740191 A1	30-10-1997
			US 6066459 A	23-05-2000
			US 5719024 A	17-02-1998
			US 5856871 A	05-01-1999
			US 5835214 A	10-11-1998
			US 5798262 A	25-08-1998
			US 6018587 A	25-01-2000
			US 5912165 A	15-06-1999
			US 5906919 A	25-05-1999
			EP 0832417 A1	01-04-1998
			IL 121425 A	13-08-2000
			JP 11503239 T	23-03-1999
			WO 9722848 A1	26-06-1997
			US 6055325 A	25-04-2000
			EP 0830564 A1	25-03-1998
			IL 121426 A	29-02-2000
			JP 11500832 T	19-01-1999
			US 6075599 A	13-06-2000
			WO 9721979 A1	19-06-1997
			US 6007996 A	28-12-1999
			US 6165734 A	26-12-2000
			US 5991028 A	23-11-1999
			US 6198532 B1	06-03-2001
			EP 0767361 A2	09-04-1997
			GR 3033470 T3	29-09-2000